OPP-300397 RABON Tetrachlorvinphos

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

MAR 6 1995

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Gardona (2nd)

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Review Section 2
Toxicology Branch II
Health Effects Division (7509C)
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TO: George LaRocca, PM #13
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Registration Division (7505C)
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THROUGH: Stephanie R. Irene Ph.D. Stephanie R. Irene 3-3-95
Acting Director, Health Effects Division (7509C)

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on, Dec. 12, 1994 to discuss and re-evaluate the weight-of-the-evidence on gardona, with particular reference to its carcinogenic potential.

Gardona was previously evaluated by the Toxicology Branch Peer Review Committee (TBRC) as a Group C with a Q_1* (Memo, dated April 14, 1988). At that time, the TBRC found that there were deficiencies in the rat study, and recommended that another rat study be performed by the Registrant. In response to this recommendation, a new rat study was submitted, and this second peer review was convened to evaluate the new rat study, and re-evaluate the weight-of-the-evidence for gardona.

The CPRC concluded that the classification of gardona should remain as Group C with a Q_1*, based on statistically significant increases in combined adenomas/carcinomas (predominantly carcinomas) in the female B6C3F1 mouse, suggestive evidence of thyroid C-cell adenomas and adrenal pheochromocytomas in the rat, mutagenicity concerns, and SAR support.

SUMMARY

Gardona was previously evaluated by the Toxicology Branch Peer Review Committee (TBRC) as a Group C with a Q_1* (Memo, dated April 14, 1988). At that time, the TBRC found that there were deficiencies in the rat study, and recommended that another rat study be performed by the Registrant. In response to this recommendation, a new rat study was submitted, and this second peer review was convened to evaluate the new rat study, and re-evaluate the weight-of-the-evidence for gardona.

In the earlier Gulf South rat study, administration of gardona in the diet to Osborne-Mendel rats was associated with statistically significant increases in thyroid C-cell and adrenal cortical adenomas in female rats. The TBRC concluded in the April 14, 1988 memo that there were deficiencies in the study design and the evidence was judged to be "equivocal".

In the new Inveresk rat study, administration of gardona in the diet to Sprague-Dawley rats was associated with an elevated incidence of thyroid C-cell adenomas and adrenal pheochromocytomas in male rats only. Neither of these increases were statistically significant by pairwise comparison to controls, but there was a statistically significant increasing trend for the adrenal tumors. The CPRC noted that although this was a different strain, the tumor sites were the same, and the results were supportive of the earlier study. The CPRC concluded that the evidence in the rat was suggestive.

In the Hazleton mouse study[1], administration of gardona in the diet to B6C3F1 mice resulted in statistically significant increases in hepatocellular adenomas, carcinomas and combined adenomas/carcinomas (with carcinomas predominant) in females, and in combined hepatocellular adenomas/carcinomas in males. In male mice there were also statistically significant increases in renal adenomas, carcinomas and combined adenomas/carcinomas. The statistically significant increases in tumors noted above, all occurred only at doses of gardona of 8000 ppm or greater, except for the combined hepatocellular adenomas/carcinomas in female mice, which also occurred at 1600 ppm.

This mouse study was previously evaluated by the TBRC, which concluded in the April 14, 1988 memo, that the highest dose tested (16,000 ppm) was excessively toxic, based on body weight gain depressions of >15%, and that the next highest dose (8000 ppm) may also have been slightly excessive, but that the 1600 ppm dose was adequate. The CPRC agreed with the previous evaluation and conclusion, that there was a statistically significant increase in hepatocellular adenomas/carcinomas in female mice, even at a dose that was not excessively toxic (1600 ppm).

[1]There were no additional studies required or submitted for the mouse.

In a second mouse study (Gulf South) also previously evaluated, study deficiencies were noted, but the TBRC then - and the CPRC now - agreed that the results were supportive of the findings in the mouse liver.

There were positive results in an in vitro chromosomal aberration assay with CHO cells in the absence of S-9 activation, suggestive evidence in a dominant lethal assay, as well as additional supportive evidence in the literature that gardona is a clastogen and positive in a mouse micronucleus assay.

Gardona is structurally related to DDVP (classified by the CPRC as a Group C with a Q_1*) and phosphamidon (Group C). Gardona can be hydrolyzed to yield a chlorinated vinyl alcohol derivative, which can then tautomerize to generate a potentially carcinogenic reactive ketone intermediate.

The CPRC agreed that the classification of gardona should remain as Group C with a Q_1*, based on statistically significant increases in combined hepatocellular adenomas/carcinomas (predominantly carcinomas) in the female B6C3F1 mouse, suggestive evidence of thyroid C-cell adenomas and adrenal pheochromocytomas in the rat, mutagenicity concerns, and SAR support.

A. Individuals in Attendance at the meetings:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Stephanie R. Irene

Reto Engler — Retired

William Burnam

Karl Baetcke

Marcia Van Gemert

Kerry Dearfield

Hugh Pettigrew

Esther Rinde

Elizabeth Doyle

Marion Copley

Yin-Tak Woo

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Byron Backus[2]

Clark Swentzel

Lori Brunsman

Lucas Brennecke[3] (PAI/ORNL)

3. Other Attendees: Bernice Fisher, Jane Smith, Steve Robbins (HED) Amber Aranda (OGC) and Dr. Roland Solecki (Federal Institute for Consumer Health Protection and Veterinary Medicine, Germany)

[2]Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

[3]Signature indicates concurrence with pathology report.

B. Material Reviewed

The material available for review consisted of DER's, one-liners, data from the literature and other data summaries prepared and/or supplied by Dr. Byron Backus, and tables and statistical analysis by Lori Brunsman. The material reviewed is attached to the file copy of this report.

C. Background Information

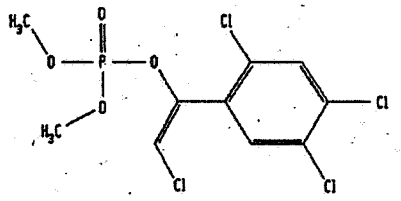

Figure 2 Gardona

CAS registry #: 961-11-5
P.C. Code #: 083701.

Gardona, also known as Rabon or Tetrachlorvinphos, was previously classified (Memo, dated April 14, 1988) as a C (possible human) carcinogen with a Q_1*, based on a dose-related increased incidence of liver tumors (combined adenomas/carcinomas) in female B6C3F1 mice.

The 1988 Peer Review Committee noted that in a carcinogenicity study (Gulf South Study) with the Osborne-Mendel rat, dietary administration of Gardona was associated with statistically significant (and dose-related) increases in thyroid C-cell adenomas and adrenal cortical adenomas in females (Table 1).

Table 1[4]

Gardona - Gulf South Osborne-Mendel Rat Study
NCI-Sponsored Study - 1978
Thyroid and Adrenal Tumor Rates# (%)
Cochran-Armitage Trend Test and Fisher's Exact Test Results

	Dietary Concentrations (ppm)			Historical controls	
	0	4250	8500	Mean	Range
Thyroid Tumors					
Males					
C-cell adenoma	2/46 (4)	2/45 (4)	3/45 (7)	(8.2)	(0-16)
Females					
C-cell adenoma	1/46 (2)*	2/50 (4)	7/46 (15)*	(20.4)	(2-31)
Adrenal Tumors					
Males					
Cortical adenoma	2/52 (4)	3/48 (6)	1/45 (2)	(14)	(2-22)
Females					
Cortical adenoma	0/50 (0)*	2/49 (4)	5/50 (10)*	(24)	(13-27)

Note: Significance of trend denoted at Control. Significance of pairwise comparisons with control denoted at Dose level.
Numbers in parentheses denote percent.
*p <0.05

It was noted by the 1988 Peer Review Committee that there were deficiencies in the study design and the evidence was judged to be equivocal. The Peer Review Committee concluded that the registrant would have to submit another rat carcinogenicity study.

[4]This table was taken from table 5 of the previous review on gardona.

D. Evaluation of Carcinogenic Evidence

1. Sprague-Dawley Rat Carcinogenicity Study. Reference: Mulhern, M., D. Robb, C.J. Perry, P. Millar, C. Atkinson: Tetrachlorvinphos: 104 week Dietary Combined Chronic Toxicity/Carcinogenicity Study in Rats: July 29, 1993. MRID Number: 429809-01.

Testing Facility: Inveresk Research International (IRI), Tranent, EH33 2NE Scotland.

Experimental Design: In this study, groups of 50 male and 50 female Charles River Sprague-Dawley rats received Gardona in their diet over a 2-year period at 0, 100, 1000 or 2000 ppm (equivalent to 0, 4, 43 and 89 mg/kg/day in males and 0, 6, 63 and 125 mg/kg/day in females, respectively).

Discussion of tumor data

There were increases in the incidences of thyroid C-cell adenomas and adrenal pheochromocytomas in male rats only. Neither of these increases were statistically significant by pairwise comparison to controls, but there was a statistically significant increasing trend for the adrenal tumors. The increased incidences of C-cell adenomas and adrenal pheochromocytomas were consistent with what was observed in the 1978 (Gulf South) NCI-sponsored study.

Tumor incidences for adrenals and thyroids from the 2-year IRI study are given in Tables 2 through 5.

Table 2

Gardona - Charles River Sprague-Dawley Rat Study
IRI - 1993

Male Adrenal Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	100	1000	2000
Pheochromocytoma, Benign (%)	4/49 (8)	2/49 (4)	6[a]/49 (12)	9/50 (18)
p =	0.018*	0.339[n]	0.370	0.125
Pheochromocytoma, Malignant (%)	0/49 (0)	0/49 (0)	1[b]/49 (2)	0/50 (0)
p =	0.503	1.000	0.500	1.000
Combined (%)	4/49 (8)	2/49 (4)	6[c]/49 (12)	9/50 (18)
p =	0.018*	0.339[n]	0.370	0.125

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[n]Negative change from control.

[a]First pheochromocytoma, benign, observed at week 76, 1000 ppm.

[b]First pheochromocytoma, malignant, observed at week 76, 1000 ppm.

[c]One animal in the 1000 ppm dose group had both a benign and a malignant pheochromocytoma.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 3

Gardona - Charles River Sprague-Dawley Rat Study
IRI - 1993

Male Thyroid C-Cell Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	100	1000	2000
Adenomas	8[a]/47	10/47	7/48	13/45
(%)	(17)	(21)	(15)	(29)
p =	0.127	0.397	0.482[n]	0.134
Carcinomas	0/47	1/47	1[b]/48	0/45
(%)	(0)	(2)	(2)	(0)
p =	0.511	0.500	0.505	1.000
Combined	8/47	11/47	8/48	13/45
(%)	(17)	(23)	(17)	(29)
p =	0.151	0.304	0.590	0.134

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[n]Negative change from control.

[a]First adenoma observed at week 74, dose 0 ppm.

[b]First carcinoma observed at week 78, dose 1000 ppm.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p \leq 0.01$.

Table 4

Gardona - Charles River Sprague-Dawley Rat Study
IRI - 1993

Female Adrenal Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	100	1000	2000
Pheochromocytoma, Benign (%)	0/48 (0)	1[a]/50 (2)	2/50 (4)	0/49 (0)
p =	0.422	0.510	0.258	1.000
Pheochromocytoma, Malignant (%)	1[b]/48 (2)	0/50 (0)	0/50 (0)	0/49 (0)
p =	0.168	0.490[n]	0.490[n]	0.495[n]
Combined (%)	1/48 (2)	1/50 (2)	2/50 (4)	0/49 (0)
p =	0.268	0.742	0.516	0.495[n]

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[n]Negative change from control.

[a]First pheochromocytoma, benign, observed at week 105, 100 ppm.

[b]First pheochromocytoma, malignant, observed at week 105, 0 ppm.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 5

Gardona - Charles River Sprague-Dawley Rat Study
IRI - 1993

Female Thyroid C-Cell Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	100	1000	2000
Adenomas	6[a]/64	4/50	5/49	4/65
(%)	(9)	(8)	(10)	(6)
p =	0.294	0.535[n]	0.564	0.362[n]
Carcinomas	0/64	0/50	1[b]/49	1/65
(%)	(0)	(0)	(2)	(2)
p =	0.120	1.000	0.434	0.504
Combined	6/64	4/50	6/49	5/65
(%)	(9)	(8)	(12)	(8)
p =	0.427	0.535[n]	0.424	0.489[n]

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.

[n]Negative change from control.

[a]First adenoma observed at week 53, dose 0 ppm.

[b]First carcinoma observed at week 105, dose 1000 ppm.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

The CPRC concluded that these results were supportive of the findings in the earlier rat study. The CPRC noted that a different sex was affected in the new study; however, it was also a different strain. Overall the CPRC characterized the evidence in the rat as suggestive.

Historical Control Data

Historical tumor data for controls: Historical control data for rat thyroid C-cell tumors in chronic studies conducted at Inveresk are given in Table 6 (no appropriate data were available for the historical incidence of adrenal pheochromocytomas).

The incidence of C-cell thyroid tumors (13/45) observed in males of the 2000 ppm dose group was slightly outside the range observed in the historical control groups (2/50 - 11/48); while the incidences observed in the other male groups were within the range (0 ppm: 8/48; 100 ppm: 11/49; 1000 ppm: 8/49).

Table 6

Historical Control Data from Inveresk
Incidences of Neoplastic and Focal Hyperplastic Lesions in
Untreated Crl:CD Sprague-Dawley Rats
Thyroid (C-Cell)

Study No.	No. examined	Focal hyperplasia	Benign tumor	Malignant tumor
593 male	50	4	2	0
593 female	50	8	1	0
609 male	48	10	11	0
609 female	47	16	3	0
504 male	49	6	5	0
504 female	49	3	2	1
090 male	46	6	5	0
090 female	49	3	2	1
140 male	50	3	8	0
140 female	50	9	7	0
728 male	49	16	4	1
728 female	49	8	4	0
059 male	47	5	5	1
059 female	48	9	3	0
411 male	49	1	3	0
411 female	50	2	2	0
944 male	48	6	2	0
944 female	45	15	1	1

Adequacy of Dosing for Assessment of Carcinogenic Potential:

In the initial review (April 1, 1994) of the study there was concern that dosing may not have been adequate. Subsequently, additional material was received, including a subchronic (13-week) rat feeding study (in MRID 433712-01), which had been conducted at IRI prior to initiation of the 2-year study.

Dietary exposure levels used in this 13-week study were 0, 100, 2000 and 5000 ppm. Male and female Sprague-Dawley rats were fed 0, 100, 2000 or 5000 ppm of gardona for 13 weeks. At 13 weeks, mean body weight gains for 2000 and 5000 ppm males were respectively 92% and 80% of their control value; for females at 2000 and 5000 ppm the values were 84 and 88% respectively. At 13 weeks, males at 2000 and 5000 ppm had significantly reduced mean plasma ChE activity (to 75% and 59% of the control value), and significantly reduced mean RBC ChE activity (to 70% and 28% of the control value). Females at 2000 and 5000 ppm had significantly reduced mean plasma ChE (to 26% and 17% of their control value) and mean RBC ChE activity (to 21% and 9% of their control value). Females at 5000 ppm had reduced mean brain ChE (to 76% of their control value), but this was not statistically significant. A noteworthy finding was that 2/10 males and 2/10 females at 5000 ppm had no measurable RBC ChE activity at 13 weeks.

The incidence of increased fat deposition in the adrenal cortex was significantly elevated in 2000 ppm (7/10) females and 5000 ppm (9/10) females. The incidence and severity of bilateral basophilic tubules of the kidneys was significantly increased in 2000 and 5000 ppm males.

General hepatocellular enlargement was noted at 2000 ppm (2/10) and 5000 ppm (7/10) in males, but not in any females. All females at 2000 and 5000 ppm had centrilobular hepatocellular hypertrophy, also present at 2000 ppm (8/10) and 5000 ppm (1/10) in males.

Thyroid follicular cell hypertrophy was present at 2000 ppm (2/10) and 5000 ppm (8/10) males and at 2000 ppm (5/10) and 5000 ppm (8/10) in females.

Mean absolute liver weights were significantly elevated in 5000 ppm females; mean covariantly adjusted (by body weight) liver weights were significantly elevated in both sexes at 2000 and 5000 ppm. Mean absolute and covariantly (body weight) adjusted adrenal weights were significantly elevated in 2000 and 5000 ppm females.

The CPRC concluded that the highest dietary exposure level (2000 ppm) in the Inveresk chronic rat study was adequate for assessment of the carcinogenic potential of gardona. This was based on depressions of mean plasma cholinesterase activity at several blood sampling times in both sexes (also observed in 1000 ppm females), and the findings from the preliminary 13-week subchronic study which utilized dietary exposure levels of 0, 100, 2000 and 5000 ppm. At 13 weeks in the subchronic study mean body weight gains in 5000 ppm males and females were respectively 80 and 88% of their control values, and males and

females at 2000 and 5000 ppm had significantly reduced mean red blood cell and plasma cholinesterase activities.

Non-neoplastic changes

Effects at 1000 and 2000 ppm in both sexes included an increased incidence and tendency to greater severity of diffuse lipidosis of the adrenal zona fasciculata, and hypertrophy of periacinar hepatocytes. Males (but not females) at 1000 and 2000 ppm had significantly increased incidences of centriacinar degenerative change of the liver; females (but not males) at 1000 and 2000 ppm had increased incidences of centriacinar fat vacuolation of the liver.

Males and females at 2000 ppm had decreased mean body weight gains at 13 weeks that were 4.4% and 10.8% less than their respective controls. Other effects noted in the study were reduced plasma cholinesterase (ChE) activity (significantly reduced in 2000 ppm males at weeks 51/52, 77/78, and 103/104 relative to their controls, but normal at week 25/26; in 1000 and 2000 ppm females it was significantly reduced at weeks 25/26, 51/52, 77/78 and 103/104). The reduction was about 50% in 1000 ppm females and about 65% at 2000 ppm. There appeared to be no effect on RBC ChE activity in 2000 ppm males, while in 1000 and 2000 ppm females there was a tendency for mean RBC ChE activity to be lower than that of their controls, but there was statistical significance only at week 77/78 (1000 ppm: 71% of the control value; 2000 ppm: 64.2% of the control value). For brain ChE there was no indication of an effect on males at 52 and 104 weeks. Highest-dose females had reduced mean brain-ChE activity at both 52 and 104 weeks (to 83.2% and 85% of control values respectively), but these reductions were not statistically significant, although there were some indications that some 2000 ppm animals may have been more sensitive than others. There were also significantly increased cholesterol levels in 2000 ppm females at weeks 77/78 and 104.

For mean liver weights for males, at 52 weeks: slight increases in liver weights were reported for the intermediate and high dose groups (8% and 10% respectively, not statistically significant) after covariance analysis. At 104 weeks: after correction for final body weight (covariance analysis) there was an apparent decrease in liver weights reported for all dose groups which received gardona (24%, $p<0.01$, low dose; 18%, $p<0.05$ and 17%, not statistically significant, high dose). This difference is considered to have arisen as a result of an atypical control value of 27.66 g compared with the background weight for this organ in this age and strain of rat of 21.78 ± 5.19 g. The weight of liver in those groups, which received gardona was considered to be normal.

For females at week 52: after correction for final body weight (covariance analysis) a slight increase in liver weights was noted in the intermediate and high dose groups (18%, $P<0.05$ and 7%, not statistically significant respectively).

2. Evidence in the Mouse[5]

Two studies were conducted with gardona in the mouse; one by Hazleton and the other by Gulf South.

In the 1980 Hazleton study in B6C3F1 mice at dose levels of 0, 17.5, 64, 320, 1600, 8000 and 16,000 ppm, there were statistically significant increases in the incidences of liver tumors in females (adenomas at 16,000 ppm, carcinomas at 8000 and 16,000 ppm and combined adenomas/carcinomas at 1600, 8000 and 16,000 ppm). The carcinoma incidence constituted over 60% of the tumor response at 8000 and 16,000 ppm (Table 7). In addition, there were statistically significant increases in tumors of the liver (combined adenomas/carcinomas) and kidney (adenomas, carcinomas and combined adenomas/carcinomas) in males at 16,000 ppm (but not at lower doses - Tables 8 and 9). The 1988 Peer Review Committee concluded that the 8000 ppm dose was adequate or slightly excessive (body weight gain depression >15%), and that the top dose of 16,000 ppm was excessive (severe liver necrosis); however the 1600 ppm dose was adequate.

The present CPRC agreed with the previous 1988 Peer Review evaluation and conclusion, that there was a statistically significant increase in hepatocellular adenomas/carcinomas in female mice, even at a dose that was not excessively toxic (1600 ppm).

In the 1978 Gulf South study there were also statistically significant increases in hepatocellular carcinomas in male B6C3F1 mice; however, deficiencies in study design were identified. The CPRC agreed (as had the 1988 Peer Review Committee) that, despite deficiencies noted in the Gulf South mouse study, the data from that study were supportive of the findings in the Hazleton study.

[5]Refer to the Peer Review Document of Gardona Memo, dated April 14, 1988, for details of these studies.

Table 7

Gardona - 1980 Hazleton Mouse Study
Female Liver Tumor Rates† (%).
Cochran-Armitage Trend Test and Fisher's Exact Test Results

	Dietary Concentration (ppm)						
Liver Tumors	0	17.5	64	320	1600	8000	16000
Carcinoma	1/119 (1)**	0/58 (0)	0/69 (0)	0/70 (0)	4/69 (6)[b]	5/66 (8)*	5/68 (7)*
Adenoma	0/113 (0)**	1/57 (2)	1/57 (2)[a]	0/59 (0)	1/57 (2)	2/56 (4)	3/58 (5)*
Adenoma and/or Carcinoma	1/119 (1)**	1/58 (2)	1/69 (1)	0/70 (0)	5/69 (7)*	7/66 (11)**	8/68 (12)**

[a]First carcinoma appeared at week 55 in the 1600 ppm group.
[b]First adenoma appeared at week 79 in the 64 ppm group.
†Number of tumor bearing animals/number of animals at risk (excluding animals that died before appearance of first tumor.

Table 8

Gardona - 1980 Hazleton Mouse Study
Male Liver Tumor Rates† (%).
Cochran-Armitage Trend Test and Fisher's Exact Test Results

	Dietary Concentration (ppm)						
Liver Tumors	0	17.5	64	320	1600	8000	16000
Carcinoma	26/113 (23)	17/58 (29)	16/58 (28)	10/51 (20)	14/55 (25)	13/60 (22)	22/59 (37)[a]
Adenoma	2/80 (2)[b]	1/37 (3)	0/42 (0)	0/35 (0)	1/39 (3)	5/47 (11)	3/47 (6)
Adenoma and/or Carcinoma	28/113 (25)*	18/58 (31)	16/58 (28)	10/51 (20)	15/55 (27)	18/60 (30)	25/59 (42)*

[a]First carcinoma appeared at week 66 in the 16000 ppm group.
[b]First adenoma appeared at week 99 in the control group.
†Number of tumor bearing animals/number of animals at risk (excluding animals that died before appearance of first tumor.

Note: Significance of trend denoted at Control. Significance of pairwise comparisons with control denoted at Dose level.
Numbers in parentheses denote percent.
*p <0.05 **p <0.01

Table 9

Gardona - 1980 Hazleton Mouse Study
Male Kidney Tumor Rates† (%).
Cochran-Armitage Trend Test and Fisher's Exact Test Results

	Dietary Concentration (ppm)						
Liver Tumors	0	17.5	64	320	1600	8000	16000
Carcinoma	0/71 (0)**	0/37 (0)	0/39 (0)	0/31 (0)	1/36 (3)[a]	1/47 (2)	9/46 (20)**
Adenoma	0/113 (0)	0/58 (0)	0/68 (0)	0/62 (0)	1/65 (2)	0/70 (0)	4/69 (6)[b]*
Adenoma and/or Carcinoma	0/113 (0)**	0/58 (0)	0/68 (0)	0/62 (0)	2/65 (3)	1/70 (1)	13/69 (19)**

[a]First carcinoma appeared at week 104 in the 1600 ppm group.
[b]First adenoma appeared at week 54 in the 16000 group.
†Number of tumor bearing animals/number of animals at risk (excluding animals that died before appearance of first tumor.

Note: Significance of trend denoted at Control. Significance of pairwise comparisons with control denoted at Dose level.
Numbers in parentheses denote percent.
*p <0.05 **p <0.01

E. Other Relevant Toxicology Information

1. Genotoxicity

There were three acceptable mutagenicity studies which satisfy the minimum testing in the three categories of gene mutation, structural chromosomal aberrations, and other genotoxic effects. In an acceptable Ames assay (MRID# 4122508), there was no evidence of a mutagenic effect at the histidine locus in any of the Salmonella typhimurium strains (TA98, TA100, TA1535, TA1537 and TA1538) used at concentrations of from 10 to 667 µg/plate in the absence of activation or at doses from 66.7-3300 µg/plate with activation. There were sufficient indications of cytotoxicity at the highest concentrations, as evidenced by thinning of the lawn in a preliminary range finding study (TA100 tested only) and in the assays, as well as reductions in numbers of revertants at highest concentrations tested. Several published studies support the negative findings in Salmonella (e.g. Mutation Res. 116: 185-216, 1983).

In an acceptable UDS assay (MRID# 42156401), rat hepatocytes were exposed for 18.2 hours to concentrations of from 5 to 40 µg/ml TCVP. At concentrations ≥ 35 µg/ml there was almost complete cytotoxicity. Preparations from cultures exposed to 10, 15, 20, 23, 25, 27 and 30 µg/ml were evaluated for evidence of UDS. There was no indication, either from mean net nuclear grain counts or percentages of cells with ≥ 5 net nuclear grains, that exposure to TCVP under these assay conditions results in UDS.

In an acceptable in vitro chromosomal aberration assay with Chinese Hamster Ovary (CHO) cells (MRID 413129-01) the exposure levels tested and evaluated were 29.9, 44.9, 59.9, 79.8 and 99.8 μg/ml (with 20-hour cell harvest, based on cell cycle delay observed in a preliminary cytotoxicity assay) in the absence of S9, and 12.5, 25, 37.6 and 75.1 μg/ml (with 10-hour harvest) in the presence of S9. There were elevated (and statistically significant) increases in chromosomal aberrations at doses ≥ 59.9 μg/ml (but not at lower doses) in the absence of S9 activation, but no indications of an effect at any dose level in the presence of S9.

Additional studies from the literature support the clastogenic activity of gardona. Using primary cultured mouse spleen cells (tested without activation only) gardona induced aberrations over a concentration range of 2.5-2.0 ug/ml (Mutation Res. 279: 165-170, 1992). Also gardona induced micronuclei in mouse bone marrow after i.p. (50 and 100 mg/kg) and oral (3000 and 6000 ppm) administration, but not by dermal (1350 mg/kg) treatment (Mutation Res. 117: 329-336, 1983).

Based on this evidence, gardona presents a mutagenicity concern via its clastogenic activity. It is noted that a dominant lethal study (MRID# 00072172) was submitted in which, although study deficiencies were noted, there was a suggestive effect of activity. In light of this evidence, a new dominant lethal study is needed to be performed.

2. Metabolism

The Agency has received and reviewed acceptable rat metabolism studies (MRID 419884-01; 412225-03) for gardona. In a preliminary study, radiolabeled material was administered orally at 250 mg/kg to groups of 2 males and 2 females; it was established that essentially no label was present in exhaled CO_2. The test material was then administered orally to individual animals in 3 groups of 5 males and 5 females at A) 5 mg/kg; B) 5 mg/kg following administration of 14 consecutive daily doses of 5 mg/kg unlabeled gardona; C) 250 mg/kg. Recovery was >95%. Most label was recovered from urine (45.68-60.01%) and feces (38.37-55.56%), with most of recovery within 48 hours of dosage. Only relatively small amounts (<0.5% of total administered) were recovered from tissues (rats were sacrificed 5 days after dosage). Trichloromandelic acid was a major metabolite present in urine (18.5-25.9% of total administered label in males, but only 9.6-12.4% in females). Desmethyl gardona was present at about 7-8% in both sexes receiving 5 mg/kg, but high-dose females excreted more of this compound (24.8% of total label) than high-dose males (11.2%). The major metabolite (>13% label) in 0-48 hr feces was trichlorophenylethanol. Unmetabolized gardona was present in limited quantity (0.3-0.4% total label) in 0-48 hr feces from the two low-dose groups, but was present at higher levels (M: 6.5%; F: 4.5% total administered label) in the high-dose group. The only other compound in feces at >2.5% total administered label was trichlorophenylethanediol.

The differences between sexes with respect to metabolism of gardona may be consistent with differences in target organ susceptibility (adrenal effects appear to be more pronounced in females). It is noted, however, that in the recent IRI 2-year study the significant dose-related trend for benign (and combined benign/malignant) adrenal pheochromocytomas was in the males, rather than the females.

3. Structure-Activity Correlations

Gardona is structurally similar to DDVP, classified by the CPRC as a Group C carcinogen with a Q_1* and phosphamidon, classified as a Group C carcinogen. All three are direct-acting alkylating agents[6] and can be hydrolyzed to yield chlorinated vinyl alcohol derivatives. The derivatives can then tautomerize to generate potentially carcinogenic reactive intermediates (e.g., dichloroacetaldehyde from DDVP; chloromethyl 3,4,5-trichlorophenyl ketone from gardona[7]).
DDVP induced forestomach squamous cell tumors in both sexes of B6C3F1 mice, and pancreatic acinar adenomas and leukemia in Fischer 344 rats. Phosphamidon produced tumors of the bladder and liver in male Sprague-Dawley rats.

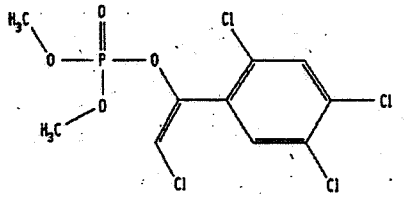

Figure 3 Gardona

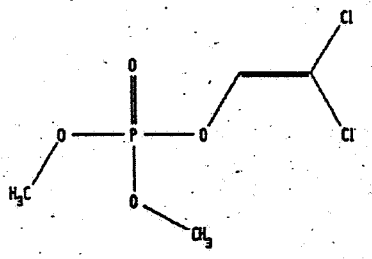

Figure 4 DDVP

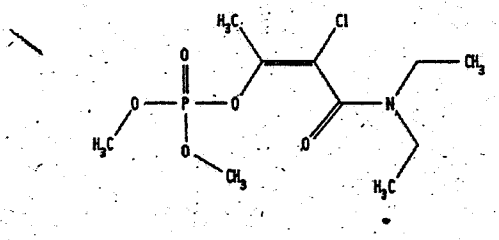

Figure 5 Phosphamidon

[6]Bedford, C.T., and Robinson, J.: Xenobiotica 2, 307 (1972).

[7]Arcos, J.C., Woo, Y.-T., and Argus, M.F.: "Chemical Induction of Cancer", Vol. IIIA, Academic Press, New York, 1982.

F. Weight of the Evidence Considerations

The Committee considered the following observations regarding the toxicology of gardona for a weight-of-the-evidence determination on its carcinogenic potential:

1. **Male and female B6C3F1 mice were fed 0, 17.5, 64, 320, 1600, 8000 or 16,000 ppm of gardona for 104 weeks. This mouse study (Hazleton) was previously evaluated by the TBRC, which concluded in the April 14, 1988 memo, that the highest dose tested (16,000 ppm) was excessively toxic, based on body weight gain depressions of >15%, and that the next highest dose (8000 ppm) may also have been slightly excessive, but that the 1600 ppm dose was adequate.**

In male mice, there was a statistically significant increase in hepatocellular adenoma/carcinoma combined at 16,000 ppm, with a statistically significant trend. In female mice, there was a statistically significant increase in hepatocellular carcinomas at 8000 and 16,000 ppm, and in combined adenoma/carcinoma at 1600, 8000 and 16,000 ppm; adenomas were statistically increased at 16,000 ppm only; there was a statistically significant trend for adenoma, carcinoma and for combined adenoma/carcinoma, as well. The present CPRC agreed with the previous 1988 Peer Review evaluation and conclusion, that there was a statistically significant increase in hepatocellular adenomas/carcinomas in female mice, even at a dose that was not excessively toxic (1600 ppm).

In males only, there was also a statistically significant increase in renal adenoma, carcinoma and adenoma/carcinoma, combined, at 16,000 ppm; there was also a statistically significant trend for adenoma, carcinoma and for combined adenoma/carcinoma, as well.

2. The CPRC agreed (as had the 1988 Peer Review Committee) that, despite deficiencies noted in the earlier Gulf South mouse study, the data from that study were supportive of the findings in the Hazleton mouse study.

3. **In the recently conducted Inveresk rat study, male and female Sprague-Dawley rats were fed 0, 100, 1000 or 2000 ppm of gardona for 104 weeks.**

There was an increased incidence (not statistically significant by pair-wise comparison) of benign pheochromocytomas of the adrenals in 2000 ppm males (9/50 vs 4/49). However, this was part of a statistically significant trend. There was also an increased incidence of male thyroid C-cell tumors at 2000 ppm (13/45 vs. 8/47), not statistically significant either by pairwise comparison or by trend test. The incidence of C-cell thyroid tumors observed in males of the 2000 ppm dose group was slightly outside the historical control range. No increased incidences of adrenal and/or thyroid tumors were evident in females.

The CPRC concluded that the highest dietary exposure level (2000 ppm) in this study was adequate, based on depressions of mean plasma cholinesterase activity at several blood sampling times in both sexes, and the findings from a preliminary (range-finding) 13-week subchronic study (described in point 3. below).

The CPRC agreed that the increased incidences of thyroid C-cell adenomas and adrenal pheochromocytomas in male rats of the recent Inveresk study were consistent with that observed in the 1978 NCI-sponsored (Gulf South) rat study.

4. **Male and female Sprague-Dawley rats were fed 0, 100, 2000 or 5000 ppm of gardona for 13 weeks. At 13 weeks, mean body weight gains for 2000 and 5000 ppm males were respectively 92% and 80% of their control value; for females at 2000 and 5000 ppm the values were 84 and 88% respectively. At 13 weeks, males at 2000 and 5000 ppm had significantly reduced mean plasma ChE activity (to 75% and 59% of the control value), and significantly reduced mean RBC ChE activity (to 70% and 28% of the control value). Females at 2000 and 5000 ppm had significantly reduced mean plasma ChE (to 26% and 17% of their control value) and mean RBC ChE activity (to 21% and 9% of their control value).**

Based on these findings, the CPRC concluded that the highest dietary exposure level (2000 ppm) in the Inveresk chronic rat study was adequate for assessing the carcinogenic potential of gardona.

5. Gardona was not mutagenic in the Salmonella (Ames) assay or in an unscheduled DNA synthesis (UDS) assay. However, in an <u>in vitro</u> chromosomal aberration assay with CHO cells there were elevated (and statistically significant) increases in chromosomal aberrations in the absence of S9, but not in the presence of S9. There was also suggestive evidence in a dominant lethal assay and additional supportive evidence in the literature that gardona is a clastogen (e.g., positive for inducing micronuclei in mouse bone marrow).

6. Gardona is structurally similar to DDVP and phosphamidon; DDVP and phosphamidon were classified by the CPRC as Group C carcinogens (DDVP with a Q_1*). Gardona can be hydrolyzed to yield a chlorinated vinyl alcohol derivative, which can then tautomerize to generate a potentially carcinogenic reactive ketone intermediate.

7. Carcinogenicity in animals -- Gardona

After a full evaluation of all of the data and supporting information regarding animal carcinogenicity, the Committee concludes that exposure to gardona resulted in an increased incidence of hepatocellular carcinomas and combined adenomas/carcinomas (predominantly malignant carcinomas) in female B6C3F1 mice. In male mice there were also increases in hepatocellular combined adenomas/carcinomas and tumors of the kidney (carcinomas, adenomas and combined adenomas/carcinomas with a large contribution from malignant carcinoma). In the male Sprague-Dawley rat there were non-significant increases in adrenal benign pheochromocytomas (significant positive trend, though) and thyroid C-cell adenomas. These latter two tumor types were consistent with the same tumor types observed in another earlier study in Osborne-Mendel rats, in which deficiencies had been noted (in response to which, the more recent Sprague-Dawley study was conducted). The mutagenicity data for gardona demonstrate clastogenic activity and this also supports a carcinogenicity concern. Analogs structurally similar to gardona (DDVP and phosphamidon) are also carcinogenic. Gardona can undergo hydrolysis, and then tautomerize to generate a potentially carcinogenic reactive ketone intermediate. The relevance of the tumor data to an evaluation of gardona's potential for human carcinogenicity is discussed elsewhere in this document.

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogen Risk Assessment" [FR51: 33992-34003, 1986] for classifying the weight of evidence for carcinogenicity.

The Peer Review Committee agreed that gardona should remain classified as a Group C - possible human carcinogen and that for the purpose of risk characterization a low dose extrapolation model be applied to the animal data for the quantification of human risk ($Q1^*$), based on the total mouse liver tumors.

The CPRC concluded that the dosing in the new (Inveresk) rat study was adequate and that the results were supportive of the findings in the earlier (Gulf South) rat study. The CPRC noted that a different sex was affected in the new study; however, it was also a different strain. Overall the CPRC did not feel that these results added significantly to the weight of the evidence, but that the results could be characterized as suggestive evidence.

The CPRC reviewed the same two mouse studies which had been reviewed in the first peer review of Gardona (there were no additional studies required or submitted for the mouse). The conclusions were essentially the same: there was evidence in the female B6C3F1 mouse (statistically significant dose-related increases in hepatocellular carcinomas and combined adenomas/carcinomas, even at a dose that was not excessive) in the Hazleton study. In addition, there were statistically significant increases in tumors of the kidney (adenomas, carcinomas and combined adenomas/carcinomas) in male mice at 16,000 ppm. (but not at lower doses). Deficiencies in the Gulf South were again noted; nevertheless, the results were considered to be supportive of the findings in the Hazleton.

Mutagenicity was discussed: the <u>in vitro</u> chromosomal aberration assay with CHO cells was positive without S-9 (but not <u>with</u>), there was suggestive evidence in a dominant lethal assay and additional supportive evidence in the literature that gardona is a clastogen and positive in the mouse micronucleus assay. Also, a new dominant lethal assay is needed to further examine possible heritable genetic effects.

Gardona is structurally similar to DDVP and phosphamidon which were classified by the CPRC as Group C carcinogens (DDVP with a Q_1^*). Gardona can be hydrolyzed to yield a chlorinated vinyl alcohol derivative, which can then tautomerize to generate a potentially carcinogenic reactive ketone intermediate.

The CPRC agreed that Gardona should remain classified as a Group C - possible human carcinogen, with a Q_1^*; however, the Q_1^* should be re-calculated using a scaling factor of 3/4 power (rather than 2/3, as was previously done) in accordance with current generally accepted guidance.

The use of a Q_1^* will be maintained for the present, but gardona may be a candidate for other risk assessment methodologies, as we become more familiar with the new guidelines.